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Prehydrated One-Electron Attachment to Azido-Modified Pentafuranoses: Aminyl Radical Formation, Rapid H-Atom Transfer and Subsequent Ring Opening

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Abstract

Methyl 2-azido-2-deoxy- α -D-lyxofuranoside (**1a**) and methyl 2-azido-2-deoxy- β -D-ribofuranoside (**2**) were prepared from D-xylose or D-arabinose, respectively. Employing ESR and DFT/B3LYP/6-31G* calculations, we investigated (i) aminyl radical (RNH•) formation and (ii) reaction pathways of RNH•. Prehydrated electron attachment to **1a** and **2** at 77 K produced transient azide anion radical (RN₃•⁻) which reacts via rapid N₂ loss at 77 K, forming nitrene anion radical (RN•⁻). Rapid protonation of RN•⁻ at 77 K formed RNH• and ⁻OH. ¹⁵N-labeled-**1a** confirmed this mechanism. Investigations employing in-house synthesized site-specifically deuterated derivatives of **1a** (e.g., CH₃ (**1b**), C4 (**1c**), and C5 (**1d**)) established that: (a) a facile intramolecular H-atom transfer from C5 to RNH• generated C5• and RNH₂. C5• formation had a small deuterium kinetic isotope effect suggesting that this reaction does not occur via direct H-atom abstraction. (b) Subsequently, C5• underwent a facile unimolecular conversion to ring-opened C4• under a reductive environment. Identification of ring-opened C4• intermediate confirms the mechanism of C5•-mediated unaltered base release associated with DNA-strand break. However, for **2**, ESR studies established thermally-activated intermolecular H-atom abstraction by RNH• from methyl group at C1. Thus, sugar ring configuration strongly influence site and pathways of RNH• mediated reactions in pentafuranoses.

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Notes

The authors declare no competing financial interests.

Supporting Information Available:

Supporting information contains the following: (a) the complete Materials and Methods Section including the synthesis and spectroscopic characterization of methyl 2-azido-2-deoxy- α -D-lyxofuranoside **1a**, its labeled derivatives **1a-1d**, and of methyl 2-azido-2-deoxy- β -D-ribofuranoside (**2**); (b) Figure S1: Fits used to estimate error limits of our simulations; (c) Figure S2: Isolation of prehydrated electron-induced RNH•, 1-Me-(C2)-ND• spectrum in **1a**, and in [¹⁵N]-**1a**; (d) Figure S3: Isolation of C5• spectrum in **1a**; (e) Figure S4: Comparison of ESR spectra of ring-opened C4• and of -OCH₂• with that of an authentic carbon-centered radical, C1'•; (f) Figure S5: ESR spectra of matched glassy samples of **1a** and of [¹⁵N]-**1a**; (g) Figure S6: ESR spectra of matched glassy samples of **1a** ([**1a**] = 10 mg/mL and 0.5 mg/mL); and (h) B3LYP/PCM/6-31+G* calculated HFCC values that were obtained employing the B3LYP/PCM/6-31+G* optimized geometries of various radicals (aminyl radical, C5•, and ring-opened C4•) in Gaussian'09.

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Graphical Abstract

Introduction

The aminyl radical, $RNH\bullet$, is an important radical in the chemistry of biomolecules such as nucleic acids.^{1–4} Nucleic acid (DNA/RNA)-base-nitrogen centered radicals, such as, exocyclic aminyl radicals ($RNH\bullet$), are often formed via deprotonation of either purine or pyrimidine base cation radicals (or holes).^{4–18} This conclusion is well-supported by electron spin resonance (ESR) spectral studies at low temperature,^{4–18} and by pulse radiolysis, flash photolysis along with product analyses studies in aqueous solutions at ambient temperature.^{19–29} In addition, our ESR studies showed that $RNH\bullet$ plays an important role in the prototropic equilibria of DNA-base delocalized π -cation radicals^{5–15} (such as, guanine cation radical,^{5–9} cytosine cation radical,^{10–12} adenine cation radical^{13–15}). Pulse radiolysis and product analyses studies established that hydroxyl radical ($\bullet OH$) can produce the same delocalized π -type $RNH\bullet$ in purines, via either $\bullet OH$ addition to the C4=C5 double bond along with subsequent water elimination or by direct abstraction.^{4,5,19–29}

We have recently observed that in azido-substituted nucleosides, in which the azido group is substituted at selective sites in pentafuranose sugar, e.g., in 3'-azidothymidine (3'-AZT),³⁰ 2'-azido-2'-deoxyuridine (2'-AZdU),³¹ 4'-azido-2'-deoxycytidine (4'-AZ-2'-dC),³² radiation-mediated prehydrated electrons³³ lead to a localized N-centered neutral aminyl radical ($T(C3')-NH\bullet$) production at the C3'-site, C2'-site, and at the C4'-site of sugar moiety respectively. The mechanism of $RNH\bullet$ formation is illustrated in scheme 1 below. Formation of nitrene anion radical ($RN\bullet^-$) occurs via prompt N_2 loss from the highly unstable azide anion radical ($RN_3\bullet^-$). Subsequently, rapid protonation of $RN\bullet^-$ leads to $RNH\bullet$ formation.³⁰

Apart from recombination to form hydrazine derivatives, $RNH\bullet$ can undergo various reactions.^{1–4,12,23,30,34–39} Our ESR studies have demonstrated that in 3'-AZT, $RNH\bullet$ abstracts a H-atom either from the methyl group at C5 in a thymine base to give the allylic $dUCH_2\bullet$ (ca. 55%) or from the C5'-atom of a proximate 3'-AZT to give $C5'\bullet$ (ca. 35%).³⁰ In cytosine derivatives, $RNH\bullet$ has been shown to undergo electrophilic addition to C5=C6 double bond.^{4,12,23,37–38} In addition, the deprotonated cytosine π cation-radical (neutral aminyl radical) tautomerizes to the iminyl (σ^-) radical.¹²

It is evident from our results that in nucleosides, e.g. in 3'-AZT, the predominant site of attack of $\text{RNH}\bullet$ is at the base and not at the pentafuranose sugar moiety.³⁰ Our results in 3'-AZT³⁰ do also show that $\text{RNH}\bullet$ causes H-atom abstraction from the sugar moiety. Therefore, to isolate and to establish the nature (i.e., intramolecular or intermolecular) of H-atom abstraction pathway by $\text{RNH}\bullet$ from the pentafuranose sugar moiety, we employ methyl 2-azido-2-deoxy- α -D-lyxofuranoside (1-Me-2-Azlyxo, **1a**) as well as methyl 2-azido-2-deoxy- β -D-ribofuranoside (1-Me-2-Azribo, **2**) as abasic model compounds (see Scheme 2) in this work. It is expected that upon one-electron reduction, 1-Me-2-Azlyxo and 1-Me-2-Azribo will similarly lead to the formation of a localized N-centered $\text{RNH}\bullet$ (i.e., 1-Me-(C2)- $\text{NH}\bullet$ and 1-Me-(rC2)- $\text{NH}\bullet$ respectively, see scheme 3).

$\text{C5}'\bullet$ is a well-established precursor of DNA strand breaks and of associated unaltered base release.^{4, 23} The ring-opened $\text{C4}'\bullet$ has been proposed as an intermediate in the well-accepted mechanism of $\text{C5}'\bullet$ mediated unaltered base release.⁴ Therefore, to test this mechanism, we report stereoselective synthesis of **1a**, **2** as well as ^{15}N -labeled 1-Me-2-Azlyxo (^{15}N -**1a**) where the terminal N-atom of the azido group was ^{15}N enriched by 99.9%. In addition, we report stereoselective syntheses of **1a** derivatives having site-specifically deuterium labeling at the methyl group (**1b**), at C4 (**1c**), and at C5 (**1d**). These syntheses allow for unambiguous electron spin resonance (ESR) characterization of 1-Me-(rC2)- $\text{NH}\bullet$ as well as 1-Me-(C2)- $\text{NH}\bullet$ produced upon radiation-mediated one-electron attachment. Our ESR studies in **1a-d** and theoretical investigations of $\text{C5}\bullet$ formation in **1a** established that: (a) 1-Me-(C2)- $\text{N}\bullet^-$ reacted with surrounding water and produced OH^- at the vicinity of a C5-H atom along with 1-Me-(C2)- $\text{NH}\bullet$ at 77 K. Via a facile H-atom transfer reaction from C5 to 1-Me-(C2)- $\text{NH}\bullet$, $\text{C5}\bullet$ was formed along with 1-Me-(C2)- NH_2 . (b) Subsequently, $\text{C5}\bullet$ converted rapidly and unimolecularly to the ring-opened $\text{C4}\bullet$. To our knowledge, this study is the first report of ESR characterization of ring-opened $\text{C4}\bullet$ under a reductive environment. On the other hand, ESR studies in **2** showed that 1-Me-(rC2)- $\text{NH}\bullet$ undergoes thermally-activated intermolecular H-atom abstraction from the methyl group at C1. These differences in the reaction of $\text{RNH}\bullet$ in **1a** and in **2** point out that the site of H-atom loss as well as its pathway ($\text{RNH}\bullet$ mediated facile intramolecular H-atom transfer or intermolecular H-atom abstraction by $\text{RNH}\bullet$) in pentafuranoses are influenced by the configurations of sugar rings.

Materials and Methods

The synthesis and spectroscopic characterization of methyl 2-azido-2-deoxy- α -D-lyxofuranoside **1a**, of the site-specifically deuterated derivatives of **1a** (e.g., CH_3 (**1b**), C4 (**1c**), and C5 (**1d**)), and of the methyl 2-azido-2-deoxy- β -D-ribofuranoside (**2**) are described in the Supporting Information. For ESR studies, methods of preparation of homogeneous glassy samples of **1a-d** and **2** in 7.5 M $\text{LiCl/D}_2\text{O}$, γ -irradiation and storage of these glassy samples including the stepwise annealing of these glassy samples are described in the Supporting Information. The ESR equipment and the experimental set-up (field calibration employing Fremy's salt, microwave power etc.) for recording the ESR spectra at 77 K along with the methods of theoretical calculations are described in the Supporting Information.

Results and Discussion

(A)

1. Synthesis of methyl 2-azido-2-deoxy- α -D-lyxofuranoside and its labeled derivatives—Various derivatives of 2-azidolyxofuranosides studied in this work (**1a–d**) were prepared employing the modified Fleet and Smith protocol⁴⁰ (Scheme 4). Briefly, D-xylose **3a** was converted in two steps to methyl 3,5-*O*-isopropylidene-D-xylofuranoside **5a** (α/β , 3:2) as reported.⁴¹ Treatment of **5a** with trifluoromethanesulfonyl chloride (TfCl) and 4-(dimethylamino)pyridine (DMAP) in methylene chloride⁴² provided 2-*O*-triflate ester **6a**. In this procedure, we obtained **6a** in better yield and purity than those following the original one⁴⁰ which had utilized triflic anhydride in pyridine. Displacement of triflate from **6a** with NaN₃ in DMF followed by removal of the isopropylidene protection group with AcOH led to the formation of methyl 2-azido-2-deoxy- α -D-lyxofuranoside **1a**.⁴⁰ Displacement of triflate from **6a** with [¹⁵N]-NaN₃ provided 2-[¹⁵N]-azido labeled **1a** ([¹⁵N]-**1a**). It is noteworthy that similar to the azido substituent in 2'-AZdU, the azido group in **1a** is in the *trans* configuration to H3 and in the *cis* configuration to H1. However, the azido group in **1a** is in the opposite geometric configuration to H4 and H5,5' in comparison to the azido group in 3'-AZT or 2'-AZdU. Treatment of D-xylose **3a** with 0.5% DCl/CD₃OD produced methyl-*d*₃ xylofuranoside **4b**, which was converted to [²H₃]-methyl 2-azido-2-deoxy- α -D-lyxofuranoside, **1b**. Subjection of 4-[²H]-D-xylose **3b** to the same synthetic sequence yielded [²H₃]-methyl 2-azido-2-deoxy- α -D-4-[²H]-lyxofuranoside, **1c**. Analogous conversion of commercially available 5-[²H₂]-D-xylose, **4c**, gave [²H₃]-methyl 2-azido-2-deoxy- α -D-5-[²H₂]-lyxofuranoside, **1d**.

2. Synthesis of methyl 2-azido-2-deoxy- β -D-ribofuranoside, **2**—The methyl 2-azidoribofuranoside⁴³ (**2**) was prepared from D-arabinose **8** employing an approach depicted in Scheme 5. Shortly, silylation of methyl D-arabinofuranoside⁴⁴ **9** with 1,3-dichloro-1,1,3,3-tetraisopropylidisiloxane (TIPDSCI) gave separable mixture of 3,5-di-*O*-TIPDS protected α and β anomers of **10**.⁴⁵ Treatment of β -anomer of **10** with TfCl/DMAP in methylene chloride⁴² provided 2-*O*-triflate ester **11**. Displacement of triflate from **11** with NaN₃ in DMF followed by removal of the silyl protection groups from **12** with TBAF afforded **2**. It is noteworthy that azido group in **2** is in *trans* configuration to H3 and *cis* configuration to H1 as in *lyxo* analogue **1a** and 2'-AZdU but it is in the opposite geometric configuration to H4 and H5,5' as compared to **1a**.

(B) Identification of Radicals by ESR

1. Characterization of neutral aminyl radical (RNH•) formed from 2-azido-2-deoxy- α -D-lyxofuranoside sugar via one-electron reduction—In this work, we investigated the one-electron reduction of methyl 2-azido-2-deoxy- α -D-lyxofuranoside (1-Me-2-Azlyxo, **1a**). From our previous work on one-electron reduction of 3'-AZT (see Scheme 1),³⁰ we expect that **1a** will produce RNH• (i.e., 1-Me-(C2)-NH•, scheme 3). In scheme 3, the N-atom at the aminyl radical site is the most abundant naturally stable ¹⁴N isotope. From our already reported assignment of T(C3')-¹⁴NH• in 3'-AZT,³⁰ it is evident that the major HFCC values in 1-Me-(C2)-¹⁴NH• are from three sources - the aminyl N-

atom, the exchangeable hydrogen at aminyl radical site (N-H/N-D) and the hydrogen at C2 (H2).

In Figure 1(A), we present the ESR spectrum (purple) due to the reaction of radiation-produced prehydrated electron with **1a** with ^{14}N substitution at 77 K. This spectrum was also recorded at 77 K in aqueous glassy (7.5 M LiCl/D₂O) samples of 1-Me-2-Azlyxo. Analysis of Figure 1(A) shows triplet from an axially symmetric anisotropic aminyl nitrogen HFCC ($A_{zz} = 41$ G, $A_{xx} = A_{yy} = 0$ G) and a large central doublet (ca. 49 G) due to the isotropic β -proton HFCC from H2- atom. The g -values also show anisotropy: $g_{\parallel} = 2.0020$ and $g_{\perp} = 2.0043$ (see Table 1 and Supporting Information Figure S1). Employing these HFCC and g -values, taking into account of the anisotropic deuterium coupling from the exchangeable NH site (schemes 1 and 2) as (3.5, 0, 6) G, using an isotropic linewidth = 10 G and a mixed (Lorentzian/Gaussian = 1) lineshape, we simulated the purple spectrum in Figure 1(A). The simulated spectrum (red) is superimposed on the experimentally obtained spectrum (purple) in Figure 1(A). Based on the excellent match of the experimental and the simulated spectra and on the HFCC values, we assign the purple spectrum in Figure 1(A) to RNH•, 1-Me-(C2)-ND•.

To make this assignment of 1-Me-(C2)-ND• unequivocal, we employed a matched sample of ^{15}N -incorporated 1-Me-2-Azlyxo, [^{15}N]-**1a**, in which the terminal N-atom of the azido group was 99.9% ^{15}N labeled. This [^{15}N]-**1a** resulted in the formation of ca. 50% 1-Me-(C2)- ^{15}ND • and 50% 1-Me-(C2)- ^{14}ND •. Therefore, subtraction of 50% of spectrum 1(A) from the experimentally obtained 77 K ESR spectrum of the matched sample of [^{15}N]-**1a** (see supporting information Figure S2) led to the blue spectrum presented in Figure 1(B).

The simulated spectrum (red, Figure 1(B)) was obtained by employing the anisotropic ^{15}N -atom HFCC (57.6, 0, 0) G and the same HFCC values of (i) anisotropic α -D (N-D), (ii) isotropic β -proton HFCC from H2-proton, along with the same mixed Lorentzian/Gaussian (1/1) linewidth, and the same anisotropic g -values. The ratio of A_{zz} value of ^{15}N (57.6 G) to that of ^{14}N (41.0 G) was found to be 1.404. This value is equal to the gyromagnetic ratio $^{15}\text{N}/^{14}\text{N}$.⁶ The gyromagnetic ratio of $^{15}\text{N}/^{14}\text{N} = 1.404$ produced the larger ^{15}N coupling but with a ^{15}N spin of $\frac{1}{2}$ instead of 1 for ^{14}N .⁶ The good fit between blue and red spectra in Figure 1(B) confirms that blue spectrum in Figure 1(B) results from 1-Me-(C2)- ^{15}ND •. These results also established that, **1a**, upon one-electron reduction at 77 K, followed the reactions shown in scheme 1 to produce the neutral aminyl radical, 1-Me-(C2)-ND•, at 77 K (scheme 6).

We note that the ^{14}N HFCC values (41, 0, 0) G of 1-Me-(C2)- ^{14}ND • (Table 1) are quite close to the corresponding HFCC values (37.5, 0, 0) G of T(C3')- ^{14}ND • from 3'-AZT.³⁰ In Table 1 below, we summarize our results on experimental hyperfine couplings and g -values of the neutral aminyl radical along with the hyperfine couplings of carbon-centered radicals from 1-Me-2-Azlyxo (**1a**), its deuterated derivatives (**1b–1d**) as well as from 1-Me-2-Azribo (**2**).

2. Subsequent reactions of 1-Me-(C2)-NH•

(I) 1-Me-(C2)-NH• does not react with the methyl moiety of OCH₃ at C1 in **1a:** 1-Me-(C2)-NH• may abstract an H-atom from the methyl moiety at C1 or from various sites (e.g., C1 to C5) of the sugar moiety in **1a**. To test the feasibility of reaction of 1-Me-(C2)-NH• with the methyl moiety at C1, neutral aminyl radicals were produced in matched samples of **1a** and [²H₃]-methyl 2-azido-2-deoxy- α -D-lyxofuranoside (**1b**, 1-CD₃-2-Azlyxo) via reactions presented in scheme 6. Subsequently, these samples were progressively annealed to ca. 160 K to follow the reactions of site-specifically generated aminyl radicals (1-Me-(C2)-NH• / 1-CD₃-(C2)-NH•). The results are presented in Figure 2 below.

It is evident from Figure 2 that samples of **1a** and **1b** resulted in very similar ESR spectra. These similarities lead to following conclusions:

- a. ESR spectra shown in Figure 2(A) clearly establish that deuteration of methyl group at the anomeric C-atom does not affect formation or hyperfine couplings of the neutral aminyl radical (see Table 1). Thus, one-electron reduction of either **1a** or **1b** leads to the same aminyl radicals having identical conformations.
- b. Annealing of these samples at 140 K for 15 min resulted in a central anisotropic doublet of ca. 21 G (see Figure 2(B)). 70% subtraction of the black spectrum in Figure 2(A) (also in supporting information Figure S3(B)) from the spectrum of **1a** sample after annealing at 145 K for 15 min and recorded at 77 K (black spectrum, supporting information Figure S3(A)) led to isolation of the ca. 21 G anisotropic doublet (supporting information Figure S3(C)). This anisotropic doublet of ca. 21 G is due to an anisotropic α -H (e.g., C5'-H).^{5,14,46-51} An established anisotropic doublet spectrum due to C5'• (anisotropic α -H (C5'-H) = ca. 21 G (see Table 1), red, supporting information Figure S3(C)) obtained via photo-excitation of adenine cation radical (A^{•+}) in the glassy (7.5 M LiCl/D₂O) sample of 3'-dAMP at the native pH (ca. 5)^{5,14,48} is superimposed on this isolated anisotropic doublet. The spectral similarities (total hyperfine splitting, lineshape, and the *g*-value at the center) of the central anisotropic doublet with those of the established anisotropic doublet C5'• spectrum (Figure S3(C)), led us to tentatively assign the central anisotropic doublet to C5• (for confirmatory C5• assignment, see Figure 3(B)). These spectral similarities further point out that the C5• formed in **1a** has the same intact furanose ring conformation as that of the C5'• found in our previous studies of DNA and RNA-nucleosides and -tides.^{5,14,46-51}
- c. Subsequent stepwise annealing of these samples to 160 K (Figures 2(C) and 2(D)) led to the simultaneous loss of line components at the wings owing to the nitrogen atom of aminyl radical and of the central doublet of ca. 21 G. At 160 K, development of a new radical spectrum (Figure 2(D)) was found to be the same for **1a** and **1b**. The *g*-value at the center of either black or blue spectrum in Figure 2(D) corresponds to that of a C-centered radical^{5,14,48} (supporting information Figure S4). From these findings, we have concluded that site of this particular radical formed owing to reaction of C5• in lyxofuranose is not located on the OCD₃ / OCH₃ group at C1. While, this radical site could be at C3, or at

C4, or at C5 of the furanoside ring, our work presented below show that each spectrum shown in Figure 2(D) results from the ring-opened C4• (see Figure 4 for this assignment).

The Spectra in Figure 2(C) also contain line components due to ring-opened C4• in Figure 2(D) as well as of the central doublet due to C5• in Figure 2(B) as shown by the dotted lines. Therefore, both spectra in Figure 2(C) result from a cohort of ring-opened C4• and C5• with furanose ring. On this basis, the spectra in Figure 2(C) are assigned to the C5• with an intact furanose ring, and the ring-opened C4• (Table 1 and Scheme 7, *vide infra*). The reaction from C5• to the ring-opened C4• is caught at this temperature.

- d. Formation of C-centered radicals in spectra 2(C) and 2(D) is further supported by annealing studies of a matched sample of [¹⁵N]-**1a**. This sample resulted in spectra having similar total hyperfine splitting, similar center, and similar lineshapes to those shown in Figures 2(B) to 2(D) (supporting information Figure S5).

(II) Intramolecular H-atom abstraction at C5 by 1-Me-(C2)-NH• leads to C5•

formation in **1a:** X-ray crystallography⁵² and NMR studies⁵³ have shown that in lyxofuranose, the 2-OH and one of the two C5-H atoms are in axial conformation. In 2-azidolixofuranoses, the 2-OH is substituted by a 2-azido group; as a result, the 2-azido and one of the two C5-H atoms are expected to be in axial conformation. Consequently, the aminyl radical N-atom would be proximate to one of the two C5-H atoms. Therefore, we posed two questions: (a) can the neutral aminyl radical (1-Me-(C2)-ND•) cause H-atom abstraction from C5, and (b) whether this H-atom abstraction reaction is intramolecular or intermolecular in nature?

In order to provide an answer to these questions, we employed matched samples of 1-CD₃-2-Azlyxo (**1b**) and [²H₃]-methyl 2-azido-2-deoxy-α-D-5-[²H₂]-lyxofuranoside (**1d**, [5,5-D,D]-1-CD₃-2-Azlyxo). Results are presented in Figure 3.

Following spectral assignments in Figures 1 and 2(A), the spectrum in blue in Figure 3(A) is assigned to [5,5-D,D]-1-CD₃-(C2)-ND• while the spectrum in black in Figure 3(A) is already assigned to 1-CD₃-(C2)-ND•. Similarities of spectra shown in Figures 1, 2(A) and 3(A) establish that deuteration at C5 in **1d** does not lead to any observable effect on spectra of these aminyl radicals. Therefore, aminyl radicals having identical conformations are produced from **1b**, **1d**, and from the remaining **1a** samples upon one-electron reduction.

Via annealing from 77 K to 140 K, the central anisotropic doublet of ca. 21 G found from **1a** and **1b** samples due to an α-proton (spectra 2(B) and the black spectrum Figure 3(B)) collapses to a singlet in the blue spectrum from **1d** (Figure 3(B) and Table 1). Comparison of aminyl radical spectrum in Figures 1, 2(A), and 3(A) with the central singlet in Figure 3(B) shows that this singlet is not associated with remaining aminyl radical, as it differs considerably in lineshape and in the *g*-value at the center.

Collapse of the central doublet of ca. 21 G to a singlet in **1d** (Figure 3(B), blue spectrum) but not in **1a** (Figure 2(B)), **1b** (Figures 2(B), 3(B)), and in [²H₃]-methyl 2-azido-2-deoxy- α -D-4-[²H]-lyxofuranoside (**1c**, [4-D]-1-CD₃-2-Azlyxo) (Figure 4(B), *vide infra*) unequivocally confirms our tentative assignment of the ca. 21 G central doublet spectrum to C5• with intact furanose ring. From the yields of C5• in 5-protonated (**1a** or **1b**) versus that of 5-D-C5• in 5-deuterated (**1d**), we estimated a small deuterium kinetic isotope effect for the formation of C5• of ca. 1.3 $\pm$ 0.3. This small value of deuterium kinetic isotope effect of RNH• mediated C5• production suggests that C5• formation does not occur via direct H-atom abstraction from C5 by the aminyl radical. Moreover, in our system of homogeneous glassy solutions at low temperature, H-atom abstraction reaction has been observed to happen near the glass transition temperature (ca. 160 – 170 K) for **2** (see Figure 5(D) (this work)), for 3'-AZT and 5'-AZT³⁰, for esters and triglycerides⁵⁴, and for amide electron adducts⁵⁵. By temperature increase from 77 to ca. 140 K, the glass does not soften significantly to allow migration of RNH• to C5 for causing H-atom abstraction. Therefore, based on these results, we conclude that in 2-azidolixofuranose, C5• formation does not occur via direct H-atom abstraction by RNH• from C5; rather, C5• production occurs via facile intramolecular H-atom transfer from C5 to RNH• forming C5• and RNH₂ (see section (C)). C5• results in the central anisotropic doublet of ca. 21 G owing to the α -hydrogen at C5 of C5• with furanose ring.

Further annealing of samples to 150 K for 15 min led to spectra shown in Figure 3(C). These spectra, in comparison to those in Figure 3(B), do not have the line components due to the aminyl radical. However, the spectrum in black in Figure 3(C) matches very well with each of the spectra shown in Figure 2(C). Following this spectral similarity and as per our assignment to the spectra shown in Figure 2(C), we assigned the central doublet of the black spectrum in Figure 3(C) to C5•. The remaining line components of the black spectrum in Figure 3(C) are due to ring-opened C4• (Figure 4, *vide infra*). Collapse of the central doublet in the black spectrum to a singlet in the blue spectrum in Figure 3(C) does: (a) unequivocally establish presence of C5• in the radical cohort in Figures 2(C) and 3(C), and, (b) support our interpretation that the spectra in Figure 2(C) and 3(C) represent the facile conversion of C5• with furanose ring to the much more thermodynamically favorable ring-opened C4• (see Table 1, Figure 4 and scheme 7). This facile ring opening occurred between 150 K and 160 K under our experimental conditions of supercooled homogeneous aqueous solution.

Similarities in ESR spectra shown in Figures 2(D) and 3(D) establish that these spectra are due to the same radical, ring-opened C4• (see Figure 4 and scheme 7, *vide infra*).

Nature of H-atom transfer from C5 leading to C5• formation: Increasing the concentration (0.5 to 10 mg/mL in 7.5 M LiCl/D₂O) of **1a** did not show any observable effect on the extent of C5• formation from spectra recorded under the same microwave power, modulation, and gain (see supporting information Figure S6). From these results, we conclude that C5• formation observed in these lyxofuranoside samples occur via facile RNH• mediated intramolecular H-atom transfer (see scheme 7).

(III) C5• in 1a and in its various derivatives converts unimolecularly to the ring-opened C4•: Our ESR studies shown in Figures 2(D) and 3(D) as well as in supporting information

Figures S4, S5(D), and S6(D) clearly demonstrate that the site of final radical formed via C5•, could be on C3, or on C4, or on C5 of the furanoside ring. To shed light on it, we employed matched samples of 1-CD₃-2-Azlyxo, **1b**, and [²H₃]-methyl 2-azido-2-deoxy- α -D-4-[²H]-lyxofuranoside (**1c**, [4-D]-1-CD₃-2-Azlyxo). Results are presented in Figure 4.

Comparing the spectra in Figure 4(A) with those in Figures 1, 2(A), and 3(A) we assign the spectrum in blue in Figure 4(A) to the neutral aminyl radical, [4-D,D]-1-CD₃-(C2)-ND•. The spectrum in black in Figure 4(A) is the same black spectrum in Figure 3(A), which is due to 1-CD₃-(C2)-ND•. Analyses of these spectra clearly show that deuteration at C4 in [4-D]-1-CD₃-2-Azlyxo did not affect the spectra of aminyl radicals.

Via annealing from 77 K to 140 K, spectra in Figure 4(B) show that the central doublet of ca. 21 G, which is due to C5•, remains unaffected in 1-CD₃-2-Azlyxo (**1b**, black) and in [4-D]-1-CD₃-2-Azlyxo (**1c**, blue) samples. Therefore, comparison of spectra in Figure 4(B) with those in Figure 3(B) clearly show that hydrogen at C4 which is at beta position to the radical site in C5•, does not show observable proton hyperfine coupling with the radical site (see section (C)).

Further annealing of samples to 150 K for 15 min led to spectra shown in Figure 4(C). Comparison of spectra in Figure 4(C) with those in Figure 2(C) and 3(C) show that only the blue spectrum from **1c** sample is a doublet but all remaining spectra in Figures 4(C) and 3(C) are similar multiplets and a cohort of ring-opened C4• and unconverted C5•. Since C5• spectrum is a doublet (ca. 21 G, *vide supra*), the ring-opened 4-D-C4• spectrum from the **1c** sample will show a doublet spectrum only if the C3-H proton shows an observable beta hyperfine coupling with the ring C4-radical site whereas the alpha hydrogen hyperfine coupling due to C4-H collapses upon deuteration at C4.

Subsequent annealing of samples to 160 K for 15 min led to spectra shown in Figure 4(D). Comparison of spectra in Figure 4(D) with those in Figures 2(D) and 3(D) show that only the blue spectrum from **1c** sample has a doublet (ca. 30 G) but all remaining spectra in Figures 2(D) and 3(D) have similar anisotropic triplets which originate from one anisotropic alpha hydrogen HFCC ((-8.0, -21.0, -30.0) G) and one near-isotropic beta hydrogen HFCC of ca. 30 G (see Table 1). As expected for the ring-opened C4•, deuterium substitution at C4 removes the anisotropic alpha H-coupling leaving only the 30 G beta proton HFCC. Further, the results presented in Figures 2 to 4 show that site-specific deuteration at C5 (Figure 3) and that at the methyl group in **1a** (Figure 2) do not affect the spectra of the final radical. On this basis, we assign the anisotropic alpha hydrogen HFCC to C4-H and the isotropic beta hydrogen HFCC of ca. -30 G to C3-H. Further annealing of **1b** sample to ca. 170 K for 15 min resulted in the black spectrum in Figure 4(E). Employing the anisotropic alpha hydrogen HFCC ((-8.0, -21.0, -30.0) G) due to C4-H, one near-isotropic beta hydrogen HFCC ((31, 28.0, 27.5) G) due to C3-H, anisotropic *g*-value (2.0044, 2.0022, 2.0052), and a mixed (Lorentzian/Gaussian = 1:1) isotropic linewidth of 5 G, a simulated spectrum (red, Figure 4(E)) was obtained and was superimposed on the experimentally recorded black spectrum. The simulated spectrum in Figure 4(E) matched well with the experimentally recorded one.

These HFCC values match very well with those predicted by theory (B3LYP/PCM/6-31+G*) for a geometry optimized ring-opened C4• system that give one anisotropic alpha hydrogen HFCC due to C4-H (−6.4, −17.8, −24.4) G and one isotropic beta hydrogen HFCC due to C3-H was obtained as 24.3 G (see section (C)). Thus, we assign the spectra in Figures 2(D), 3(D), 4(D), 4(E), as well as in supporting information Figures S4, S5(D), and S6(D) to ring-opened C4•. The C5• to C4• conversion is very facile as it happened between 150 K and 160 K (i.e., within 10 K) under our experimental conditions that employ supercooled homogeneous aqueous solutions at low temperatures.

C5• converts unimolecularly to ring-opened C4•: Increasing the concentration (0.5 to 10 mg/mL in 7.5 M LiCl/D₂O) of **1a** did not show any observable effect on the extent of ring-opened C4• formation from spectra recorded under the same conditions (see supporting information Figure S6). From these results, we conclude that the C5• with furanoside ring converts unimolecularly to ring-opened C4• (Scheme 7).

Summary of results obtained from 1a and its derivatives: Our ESR spectral studies using matched samples of **1a–d** as well as using various concentrations of **1a** clearly established that via facile 1-Me-(C2)-ND• mediated intramolecular H-atom transfer from C5, radiation-produced 1-Me-(C2)-ND• converts to C5• with intact furanose ring and 1-Me-(C2)-NH₂. Subsequently, this C5• undergoes facile unimolecular conversion to the ring-opened C4• (Scheme 7).

(IV) Intermolecular H-atom abstraction from -OCH₃ at C1 by 1-Me-(rC2)-NH• leads to -OCH₂• formation in methyl 2-azido-2-deoxy-β-D-ribofuranoside (1-Me-2-Azribo, 2): The ribofuranose ring in 2'-azidonucleosides/tides is predominantly in the C3-endo conformation.⁵⁶ Therefore, we posed two questions: (a) In C3-endo conformation, would proximity of H-atom of methoxy group at C1 to the aminyl radical site in 1-Me-(rC2)-NH• allow for H-atom abstraction? And, (b) if H-atom abstraction from the methoxy group at C1 does occur, whether it occurs intramolecularly or intermolecularly? Our work employing 1-Me-2-Azribo (**2**) sample provides answer to these questions and the results are presented in Figure 5 below.

Comparison of the experimentally recorded spectra (black) presented in Figures 5(A) to 5(C) with those presented in Figures 1, 2(A), 3(A), and 4(A) points out that each black spectrum in Figures 5(A) to 5(C) show the line components of the triplet due to an axially symmetric anisotropic aminyl nitrogen HFCC ($A_{zz} = 43.0$ G, $A_{xx} = A_{yy} = 0$ G (Figure 5(A) and (B), $A_{zz} = 41.6$ G, $A_{xx} = A_{yy} = 0$ G (Figure 5(C))) at the wings and a large central doublet (ca. 46.2 G (Figure 5(A)), ca. 49.2 G (Figure 5(B)), and ca. 50 G (Figure 5(C))) due to the isotropic β-proton HFCC from H2. Each of the black spectra in Figure 5(A) to (C) show the same anisotropic g -values ($g_{||} = 2.0020$ and $g_{\perp} = 2.0043$) that are identical to those of 1-Me-(C2)-NH•/1-Me-(C2)-ND• in Figure 1. Employing these HFCC and g -values, taking into account of the anisotropic deuterium coupling from the exchangeable NH site (schemes 2 and 3) as (3.5, 0, 6) G, using an isotropic linewidth = 10 G and a mixed (Lorentzian/Gaussian = 1) lineshape, we simulated the black spectrum in Figures 5(A), 5(B), and in 5(C). Based on the excellent match of the experimental and the simulated spectra (red) and on the HFCC values, we assign the black spectrum in Figure 5(A), 5(B), and 5(C) to RNH•, 1-Me-(rC2)-ND•/1-

Me-(rC2)-NH•. We also note here that the anisotropic nitrogen HFCC and the isotropic β -proton HFCC from H2-atom show slight change upon warming from 77 to 150 K owing to relaxation of the aminyl radical. Moreover, the HFCC values of the anisotropic aminyl nitrogen ($A_{zz} = 41$ G, $A_{xx} = A_{yy} = 0$ G) and the HFCC values of the isotropic β -C2-H proton (ca. 49 G) of 1-Me-(C2)-NH• are identical to those of the anisotropic aminyl nitrogen in 1-Me-(rC2)-NH•.

Upon annealing the sample to 160 K, the experimentally recorded spectrum at 77 K (black, Figure 5(D)) showed an overall anisotropic triplet (1:2:1); the g -value at the center of this spectrum corresponds to that of a C-centered radical^{5, 14, 46–51} (supporting information Figure S4). This sort of anisotropic 1:2:1 triplet originates owing to two α -H couplings in -OCH₂•.^{50,57} The spectrum of -OCH₂• has been simulated using the ESR parameters: 1 α H ($A_{xx}, A_{xy}, A_{yy}, A_{zz} = 24.3, 11.1, 20.9, 21.6$) G, 1 α H ($A_{xx}, A_{xy}, A_{yy}, A_{zz} = 9.5, 3.1, 31.5, 20.7$) G, ($g_{xx}, g_{yy}, g_{zz} = 2.0037, 2.0047, 2.0019$), and with a mixed (1:1) Lorentzian/Gaussian linewidth (5.0, 5.0, 5.5) G (for determination of the non-diagonal tensor element A_{xy} , see “Electron Spin Resonance” in Materials and Methods in the supporting information. We note here that the diagonal tensor elements A_{yy} and A_{zz} are experimentally determined (see Table 1)). The simulated spectrum (red, Figure 5(D)) of -OCH₂• matches the experimental spectrum (black, Figure 5(D)) quite well. Based upon these results, we have assigned this anisotropic triplet found in -OCH₂• spectrum to the two α -H hyperfine couplings from the -OCH₂ group (scheme 8).

The -OCH₂• formation was found to depend on the concentration of **2**. No -OCH₂• formation was observed at [**2**] = 0.9 mg/mL. Thus, contrary to lyxofuarnosides **1a–d**, results in Figure 5 established that: (a) 1-Me-(rC2)-ND•/1-Me-(rC2)-NH• mediated -OCH₂• formation occurred via facile (i.e., upon warming from ca. 150 K to ca. 160 K) intermolecular H-atom abstraction in high concentration (e.g., 5.4 mg/mL) of **2**; (b) the aminyl radical site in C3-endo conformation of 1-Me-(rC2)-ND• (or, 1-Me-(rC2)-NH•) cannot lead to -OCH₂• either via intramolecular H-atom abstraction or via 1-Me-(C2)-ND• mediated facile intramolecular H-atom transfer from the sterically inaccessible methoxy group at C1 in its β -anomeric form (scheme 8).

Comparison of results from 1a and its derivatives with those from 2: In summary, 1-Me-(C2)-ND•/1-Me-(C2)-NH• formed in α -anomeric form of 2-azidoloxofuranosides (parent (**1a**) and derivatives of **1a** (¹⁵N-**1a**], **1b**, **1c**, and **1d**)), owing to its proximity to one of the two C5-H atoms to the aminyl nitrogen radical site, lead to the formation of C5• via facile 1-Me-(C2)-ND• mediated intramolecular H-atom transfer. On the other hand, 1-Me-(rC2)-ND•/1-Me-(rC2)-NH• in β -anomeric ribofuranoside **2** leads to -OCH₂• production via intermolecular H-atom abstraction.

(C) Studies employing density functional theory (DFT)

Aminyl radical (1-Me-(C2)-ND•/1-Me-(C2)-NH•) formation in 2-azidoloxofuranoside via nitrene anion radical intermediate (scheme 6)—At first, we performed geometry optimization of the nitrene anion radical (scheme 6) in the presence of one water molecule including the effect of full solvation employing B3LYP/PCM/

6-31+G* method (see Materials and Methods, supporting information). Our calculations show that formation of the aminyl radical is instantaneous by protonation of the nitrene anion radical from the water molecule producing $\cdot\text{OH}$ (scheme 6 and Figure 6). This validates our observation of robust aminyl radical formation at 77 K in azidosugars (lyxo and ribofuranosides, this work as well as in our previous work³⁰ on 3'-AZT and 5'-AZT). The spin density distribution plot of the aminyl radical shown in Figure 6 establishes that the spin (or, the unpaired electron) is localized on the aminyl nitrogen and is a π -type radical. The B3LYP/PCM/6-31+G* calculated ^{14}N HFCC values were obtained as (42.0, 1.1, 1.3) G. The beta C2-H and exchangeable alpha N-H HFCC value were predicted as 44 G and (-39.2, -1.1, -26.9) G respectively. These theoretically calculated HFCC values agree very well with the corresponding experimentally obtained ones (see Table 1): ^{14}N HFCC = (41.0, 0, 0) G, beta C2-H HFCC = 49 G, and A_{zz} component of alpha NH HFCC = 28 G (see Figure 1 and its discussion). Without constraining this dihedral angle, the beta C2-H HFCC value was found to be 0.2 G i.e., the C2-H beta proton appeared to be in the nodal plane of the aminyl radical site p -orbital. Therefore, to match the experimentally observed beta C2-H HFCC value, beta C2-H HFCC was calculated by constraining the dihedral angle H2-C2-N2-H = 123 deg. We note here that rotation of N-H bond around N2-C2 does not change the total energy of the aminyl radical considerably.

C5• formation in 2-azidolixofuranoside—The small deuterium kinetic isotope effect found on C5• formation in 5-deuterated **1a** (see Figure 3) suggests that C5• formation does not occur via direct H-atom abstraction from C5 by 1-Me-(C2)-ND•. To produce C5•, we moved a proton from C5 to 1-Me-(C2)-ND• and performed full geometry-optimization of the structure (Figure 6, C5•). The spin density distribution plot of C5• shows that the unpaired spin is primarily located on C5-H thereby resulting in substantial anisotropic alpha proton HFCC values of (-3.0, -17.1, -27.0) G and a very small isotropic C4-H beta HFCC value of 2.4 G. Experimentally, the ESR spectrum due to C5• was found to be an anisotropic doublet of ca. 21 G (see Figure 2(B) and 3(B)) due to the anisotropic α -H HFCC at C5. As per our previous work on C5'• formation via photoexcitation of $\text{A}^{\bullet+}$ in dAdo⁴⁸ and in Ado¹⁴, the theoretically calculated (ca. 16 G) and the experimentally obtained (ca. 21 G) anisotropic C5'-alpha proton HFCCs are in good agreement. The very small isotropic beta HFCC due to C4-H proton justifies the anisotropic doublet experimental spectrum and points out that C4-H proton does not couple with the radical site p -orbital. As a result, the C4-proton in C5• should be in the nodal plane of the p -orbital at the radical site. Furthermore, the experimental observations in case of C5• formation - (a) the small value of deuterium kinetic isotope effect and (b) the intramolecular and facile nature at low temperature (between 77 and 140 K) suggest that the rapid H-atom transfer process from C5 to the aminyl radical (1-Me-(C2)-ND•/1-Me-(C2)-NH•) is not a simple H-atom abstraction and does likely involve the $\cdot\text{OH}$ that was formed initially via protonation of $\text{RN}^{\bullet-}$ by the surrounding water (see Figure 6). This rapid H-atom transfer could likely be a proton-coupled electron transfer (PCET) process.

Assignment of ring-opened C4• and relative stabilization as well as relative free energies of various radicals—To strengthen the assignments of ESR spectra in Figures 2(D), 3(D), 4(D), 4(E), as well as in supporting information Figures S4, S5(D), and

S6(D) to ring-opened C4• (scheme 7), we have performed theoretical calculations. Employing the B3LYP/PCM/6-31+G* method implemented in the Gaussian'09 set of programs,⁴² geometries of these radicals were optimized and energies were calculated. These optimized geometries, their spin densities, as well as their relative stabilization energies are presented in Figure 6.

Relative stabilization energies as well as relative free energies of aminyl radical, C5• and ring-opened C4• with respect to that of parent aminyl radical (i.e., stabilization energy and free energy of aminyl radical were set as 0.0 kcal/mol), were obtained in kcal/mol as -5.3 (-5.9) and -14.5 (-18.3) respectively. The relative free energy values are mentioned above in parenthesis. Spin density distributions shown in Figure 6 along with above-mentioned relative energies clearly establish that ring-opened C4• should be the thermodynamically most stable radical and theoretically predicted HFCC values of α -H at C4 and β -H at C3 of ring-opened C4• should agree very well with the experimentally obtained hyperfine couplings.

To validate the above theoretical prediction regarding HFCC of ring-opened C4•, the optimized geometry of ring-opened C4• obtained by Spartan'14⁵⁸ was used to calculate HFCC values employing DFT/B3LYP/6-31G* method in the gas phase as implemented in Gaussian'09 program set⁵⁹. For ring-opened C4•, theoretically calculated one anisotropic alpha hydrogen HFCC due to C4-H was obtained as (-6.4, -17.8, -24.4) G and one isotropic beta hydrogen HFCC due to C3-H was obtained as 24.3 G. The experimental HFCC values were: anisotropic alpha hydrogen HFCC ((-8, -21, -30) G) due to C4-H and one isotropic beta hydrogen HFCC (ca. 30 G) due to C3-H (*vide infra*, Figure 4). The good match between the experimental and theoretical HFCC values further confirm our assignment of ESR spectra due to ring-opened C4•.

Conclusions

The most significant aspects of this work are:

1. Unequivocal characterization of radicals: Radicals produced by prehydrated electron attachment to azido substituted lyxofuranosides and ribofuranosides are found to be aminyl radicals in which the radical site is localized to the aminyl nitrogen atom. This work highlights the importance of employing isotopically substituted azido (D, ¹⁵N) derivatives and theory to achieve unequivocal characterization of various radical species.
2. Reactions of aminyl radicals are dependent on the configuration of sugar moiety: We have employed various sugar site-specific isotopically substituted (D, ¹⁵N) methyl lyxofuranoside derivatives in this work. These compounds helped us to establish that the aminyl nitrogen radical undergoes aminyl radical mediated facile intramolecular H-atom transfer in α -anomeric lyxofuranoside (**1a-d**) forming C5•. On the other hand, for the β -anomeric ribofuranoside (**2**), which is in a C3-endo conformation, the aminyl radical site cannot sterically access either the C5-H atoms or the H-atoms of methoxy group at C1 of the same molecule. As a result, the aminyl radical of the β -anomeric ribofuranoside undergoes

intermolecular H-atom abstraction to form the methoxy radical, $-\text{OCH}_2\bullet$. The sites of H-atom loss along with the pathways of the reactions (aminyl radical mediated facile intramolecular H-atom transfer vs. intermolecular H-atom abstraction) of aminyl radicals allow us to identify the configurations of these sugar radicals in various pentafuranoses employing ESR spectroscopy and theoretical calculations.

3. Implication of conversion of $\text{C5}'\bullet$ to ring-opened $\text{C4}'\bullet$ on the pathways DNA damage: Dizdaroglu and von Sonntag performed GC-MS studies of γ -irradiated N_2O -saturated oxygen free aqueous solution of thymidine^{4,60} and showed that the release of thymine base is correlated with the formation of 2,4-dideoxypentodialdose (scheme 9). Their GC-MS studies of γ -irradiated N_2O -saturated oxygen free aqueous solution of glucose reported 5-deoxy-xylo-hexodialdose formation.^{61, 62}

The mechanism of formation of 2,4-dideoxypentodialdose was proposed to occur after formation of $\text{C5}'\bullet$ through the ring-opened $\text{C4}'\bullet$ intermediate (scheme 9). Our work presents the first evidence of the facile conversion of $\text{C5}\bullet$ to ring-opened $\text{C4}\bullet$, and, importantly we are the first to identify the ring-opened $\text{C4}\bullet$ intermediate. As a result, our work (scheme 7) confirms the mechanism presented in scheme 9 which shows that $\text{C5}'\bullet$ leads to the release of unaltered base in irradiated DNA-model system and DNA itself.^{4, 60} Moreover, for γ - and for ion-beam irradiated (77 K irradiation) DNA samples with both hole and electron scavengers which isolates the sugar-phosphate backbone radicals,^{5, 50, 63} our results indicate that at 77 K, the spectrum due to $\text{C5}'\bullet$ contributes substantially (ca. 30 to 40%) to the spectrum of sugar-phosphate backbone radical cohort.^{5, 50} $\text{C5}'\bullet$ formation was also previously observed via photoexcitation of guanine and adenine cation radicals in DNA and RNA-models;^{5,14,46–51} however, owing to the presence of $\text{K}_2\text{S}_2\text{O}_8$ as the radiation-produced electron scavenger in our previous studies^{5,14,46–51}, we could not observe this ring opening reaction of $\text{C5}'\bullet$ as it was rapidly oxidized by $\text{K}_2\text{S}_2\text{O}_8$.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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61. In addition, formation of 5-deoxy-xylo-hexodialdose was observed in γ -irradiated N₂O-saturated solution of glucose (see ref. 62). Our work (scheme 7) along with 2,4-dideoxypentodialdose formation in N₂O-saturated and γ -irradiated aqueous solution of thymidine (scheme 9) would propose the following mechanism. Firstly, the H-atom abstraction from C-6 of glucose by

radiation-produced $\bullet\text{OH}$ leads to $\text{C6}\bullet$ production; subsequently, $\text{C6}\bullet$ is converted facilely and unimolecularly to the ring-opened $\text{C5}\bullet$ which would lead to 5-deoxy-xylo-hexodialdose formation via similar pathways shown in schemes 7 and 9.

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Figure 1.

ESR spectrum at 77 K of 1-Me-(C2)-ND• which was formed via radiation (⁶⁰Co γ) - produced prehydrated electron (absorbed dose = 500 Gy) at 77 K in aqueous glassy (7.5 M LiCl/D₂O) sample of (A) unlabeled 1-Me-2-Azlyxo (**1a**), purple color, and of (B) [¹⁵N]-**1a**, blue color. The simulated spectra (red) (for simulation parameters, see text) are superimposed on the experimentally recorded spectra. All spectra were recorded at 77 K. The three reference markers (open triangles) in this Figure and in other Figures show position of Fremy's salt resonances with the central marker at $g = 2.0056$. Each of these markers is separated from each other by 13.09 G.

Figure 2.

ESR spectra obtained from matched samples (5 mg / ml in 7.5 M LiCl/D₂O (pH/pD ca. 5)) of **1a** (black) and of **1b** (blue): (A) 1-Me-(C2)-NH• formation via radiation-produced (absorbed dose = 500 Gy) prehydrated electron attachment at 77 K. Spectra (B) to (D) demonstrate subsequent reactions of 1-Me-(C2)-NH• via annealing from 77 K to ca. 160 K leading to the formation of ring-opened C4• via C5•. Spectra (C) contain line components due to ring-opened C4• in Figure 2(D) and of central doublet due to C5• in Figure 2(B) as shown by dotted lines (see scheme 7, *vide infra*). All spectra were recorded at 77 K.

Figure 3. ESR spectra obtained from matched samples (5 mg / ml in 7.5 M LiCl/D₂O (pH/pD ca. 5)) of **1b** (black) and of **1d** (blue) showing neutral aminyl radical formation via (A) ⁶⁰Co γ-radiation-produced (absorbed dose = 500 Gy) prehydrated electron attachment at 77 K. Spectra (B) to (D) represent the subsequent reactions of the aminyl radical via progressive annealing from 77 K to ca. 160 K. ESR Spectra shown in (A) to (D) were recorded at 77 K.

Figure 4.

ESR spectra obtained from matched samples (5 mg / mL in 7.5 M LiCl/D₂O (pH/pD ca. 5)) of 1-CD₃-2-AzLyxo (**1b**, black) and of ([4-D]-1-CD₃-2AzLyxo (**1c**, blue) showing neutral aminyl radical formation via (A) ⁶⁰Co γ -radiation-produced (absorbed dose = 500 Gy) prehydrated electron attachment at 77 K. Spectra (B) to (E) represent the subsequent reactions of the aminyl radical via progressive annealing from 77 K to ca. 170 K. ESR Spectra shown in (A) to (E) were recorded at 77 K. Spectrum (E, red) is the simulated spectrum of ring-opened C4• (for simulation parameters, see text).

Figure 5.

ESR spectra (black) obtained from **2** (5.4 mg / mL in 7.5 M LiCl/D₂O (pH/pD ca. 5)) showing neutral aminyl radical formation via (A) ⁶⁰Co γ-radiation-produced (absorbed dose = 500 Gy) prehydrated electron attachment at 77 K. Spectra (B) to (D) represent the subsequent reactions of the aminyl radical via progressive annealing from 77 K to ca. 160 K. ESR Spectra in black shown in (A) to (D) were recorded at 77 K. Spectrum (D, black) is assigned to -OCH₂• formed via intermolecular H-atom abstraction from the methoxy group at C1 by 1-Me-(rC2)-ND•. The simulated spectrum (red) is superimposed on each experimentally recorded spectrum (black) (for simulation parameters, see text).

Figure 6. B3LYP/PCM/6-31+G* Calculated spin densities and relative stabilization energies (in kcal/mol) of the aminyl radical, C5•, and the ring-opened C4• from **1a**.

Scheme 1.

Neutral aminyl radical, T(C3')-NH•, formation from 3'-AZT via one-electron reduction due to radiation-produced prehydrated electrons.³⁰

Scheme 2.

Structures of methyl 2-azido-2-deoxy-α-D-lyxofuranoside (1-Me-2-Azlyxo, **1a**) and methyl 2-azido-2-deoxy-β-D-ribofuranoside (1-Me-2-Azribo, **2**). The atom numbering schemes are also presented here.

Scheme 3.

Structures of $\text{RNH}\bullet$ - $\text{T(C3')-}^{14}\text{NH}\bullet$ from 3'-AZT, $\text{1-Me-(C2)-}^{14}\text{NH}\bullet$ from **1a**, and $\text{1-Me-(rC2)-}^{14}\text{NH}\bullet$ from **2**. Please note that in nucleoside systems including, for example, the nucleoside aminyl radical ($\text{T(C3')-}^{14}\text{NH}\bullet$), the sugar atoms are denoted with a prime to distinguish them from the numbered atoms of the nitrogenous bases.

Scheme 4.

Reagents (a) 0.5% HCl/CH₃OH or 0.5% DCl/CD₃OD; (b) (CH₃)₂CO/conc. H₂SO₄; (c) TfCl/DMAP/CH₂Cl₂; (d) NaN₃ or [¹⁵N]-NaN₃/DMF; (e) AcOH/H₂O (7:3)

Scheme 5.

Reagents (a) 0.5% HCl/CH₃OH; (b) TIPDSOCl/pyridine; (c) TfCl/DMAP/CH₂Cl₂; (d) NaN₃/DMF; (e) TBAF

Scheme 6.

Formation of neutral aminyl radical (1-Me-(C2)-NH•) from 1-Me-2-Azlyxo, **1a**, via one-electron reduction due to radiation-produced prehydrated electrons.

Scheme 7.

Facile unimolecular formation of thermodynamically stable ring-opened C4• via ring opening of a C5• intermediate in [5,5-D,D]-1-CD₃-2-Azlyxo (**1d**). C5• production is shown to occur via facile intramolecular H-atom transfer at C5 by the neutral aminyl radical, [5,5-D,D]-1-CD₃-(C2)-NH•.

Scheme 8.

Facile intermolecular formation of thermodynamically stable $\text{-OCH}_2\bullet$ with intact ribofuranose ring in 1-Me-2-Azribo (**2**) via H-atom abstraction by 1-Me-(rC2)- $\text{NH}\bullet$.

Scheme 9.

Formation of 2,4-dideoxypentodialdose and thymine base in N_2O -saturated and γ -irradiated aqueous solution of thymidine.^{4, 60}

Table 1

Hyperfine couplings for radicals and g -value of the aminyl radicals along with the hyperfine couplings of carbon-centered radicals found in 1-Me-2-Azlyxo (**1a**) and its derivatives as well as in 1-Me-2-Azribo (**2**)

Compound	1-Me-(C2)-ND•	C5•	C4•	-OCH ₂ •	g -value of the aminyl radical (exp)
1-Me-2-Azlyxo (1a)	H2(1 β -H) 49 N (0, 0, 41.0) ^a	C5-H (α -H) ca. 21	C4-H (α -H) ca. (-8, -21, -30) C3-H (β -H) ca. 30	-	$g_{ } = 2.0020$, $g_{\perp} = 2.0043$
1-CD ₃ -2-Azlyxo (1b)	H2(β -H) 49 G N (0, 0, 41.0) ^a	C5-H (α -H) ca. 21 G	C4-H (α -H) ca. (-8, -21, -30) C3-H (β -H) ca. 30	-	
[5,5-D,D]-1-CD ₃ -2-Azlyxo (1d)	H2(β -H) 49 N (0, 0, 41.0) ^a	$_b$	C4-H (α -H) ca. (-8, -21, -30) C3-H (β -H) ca. 30	-	
[4-D]-1-CD ₃ -2-Azlyxo (1e)	H2(β -H) 49 N (0, 0, 41.0) ^a	C5-H (α -H) ca. 21	C3-H (β -H) ca. 30 G ^c	-	
1-Me-2-Azribo (2)	H2(β -H) 46.2 to 50 N (0, 0, 41.6 to 43) ^a	-	-	1 α H ($A_{zz} = 21.6$, $A_{yy} = 20.9$), 1 α H ($A_{zz} = 20.7$, $A_{yy} = 31.5$) ^d	

^aThe HFCC value of N-D (α -deuteron) = (3.5, 0, 6) G is taken from ref. 30

^bThe anisotropic doublet of ca. 21 G due to C5-H (α -H) collapses to a singlet upon deuteration at C5 (see Figure 3).

^cThe anisotropic α -H due to C4-H collapses upon deuteration at C4 (see Figure 4).

^dThe experimentally obtained A_{zz} and A_{yy} values of hyperfine coupling constant.